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MUTAGENIC EVALUATION OF  
COMPOUND FDA 73-4

Potassium Sorbate

Mutagenic Evaluation of Compound FDA 73-4 (Potassium Sorbate)

11/25/74

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LBI PROJECT #2468

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MUTAGENIC EVALUATION OF  
COMPOUND FDA 73-4

Potassium Sorbate

SUBMITTED TO

FOOD & DRUG ADMINISTRATION  
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
ROCKVILLE, MARYLAND

SUBMITTED BY

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EVALUATION SUMMARY

Compound FDA 73-4, Potassium Sorbate, did not exhibit any genetic activity .  
in the tests used in this evaluation.



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DATE: November 25, 1974

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Mutagenic Evaluation of Compound FDA 73-4

I. OBJECTIVE

The objective of this study was to assess the genetic activity of the test material in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

II. MATERIALS

A. Test Material

Potassium Sorbate

Pfizer #72683

B. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung and testes of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males.

C. Indicator Organisms

The indicator organisms used for all tests are described below:

- Saccharomyces cerevisiae, strain D4:  $\frac{\alpha}{a}$ ,  $\frac{ade\ 2-2}{ade\ 2-1}$ ,  $\frac{try\ 5-12}{try\ 5-27}$
- Salmonella typhimurium, strains:
  - TA-1535; hisG, uvrB, rfa (missense mutation)
  - TA-1537; hisC, uvrB, rfa ( - frameshift mutation)
  - TA-1538; hisD, uvrB, rfa ( + frameshift mutation)

D. Reaction Mixture

The following reaction mixture was employed in the activation tests:



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<u>Component</u>		<u>Final Concentration/ml</u>	
1.	TPN (sodium salt)	6	μM
2.	Isocitric acid	49	μM
3.	Tris buffer, pH 7.4	28	μM
4.	MgCl <sub>2</sub>	1.7	μM
5.	Isocitric dehydrogenase	1.0	Unit
6.	Tissue homogenate or cell fraction	72	mg

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

#### E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1  
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>ASSAY</u>	<u>CHEMICAL<sup>a</sup></u>	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY<sup>b</sup></u>
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrosofluorene	Dimethylsulfoxide <sup>c</sup>	FS
	Quinacrine or	Water or saline	FS
	Quinacrine mustard		
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide <sup>c</sup>	FS

<sup>a</sup> Concentrations given in the Results Section.

<sup>b</sup> BPS = base-pair substitution; FS = frameshift.

<sup>c</sup> Previously shown to be non-mutagenic, see Appendix.

### III. METHODS

#### A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against strains TA-1537 and D4 over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.



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If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

## B. Plate Tests

Only three bacteria strains were tested in qualitative plate tests. In the non-activation procedure, approximately  $10^9$  cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. The results were scored as + or -. Concentrations of the positive control compounds are listed in the Results Section.

## C. Suspension Tests

### 1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of  $1 \times 10^9$  cells/ml and  $5 \times 10^7$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in 30 ml plastic tissue culture flasks. Cells plus appropriate volume(s) of the test chemical were added to the flasks to give a final volume of 2 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the flasks were set in ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium. Samples from a  $10^{-1}$  dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

### 2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests except that the cell densities were increased approximately five-fold for working stock suspensions. Measured amounts of the test and



control chemicals plus 0.25 ml of the stock cell suspension were added to a 30 ml plastic tissue homogenate. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

#### D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

##### 1. Mice

Male mice (sufficient to provide the necessary quantities of organs for testes, lung and liver homogenates) were killed by cranial blow, decapitated and bled. The three organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

##### 2. Rats

The same procedures as described for mice were used for this mammal.

##### 3. Primates

The liver, lungs and testes were aseptically removed from freshly killed adult male rhesus (*M. mulatta*) monkeys. Each organ was cut into a number of pieces each sufficient for one week's studies. The tissues were labeled and frozen at -80°C until needed. Tissue homogenates and 9,000 x g supernatants were prepared as described for mice.

#### E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, in bound data books. Information necessary for identification of the specific experiment as well as the presence of any contaminant micro-organisms was recorded with each set of plate counts. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated.



Frequencies were mechanically calculated and double checked. All data presented in the Results Section of this report consists of the actual sum of all raw data plate counts and only the frequencies are calculated figures.



IV. SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND: FDA 73-4  
Potassium Sorbate  
Pfizer #72683
2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY  
OF THE TEST CHEMICAL UNDER TREATMENT  
CONDITIONS: Stock solutions of this chemical were prepared in  
phosphate buffer, pH 7.4. The compound appeared completely  
soluble at treatment concentrations.
3. OTHER COMMENTS:



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V. TOXICITY AND DOSAGE DETERMINATIONSCOMPOUND FDA 73-4

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D4

TA-1537

	Dose No.	% Concentration	% Concentration
Range of concentrations of the test compound used to determine the 50% survival level	1	1	0.1
	2	2	0.5
	3	3	1.0
	4	4	2.5
	5	5	5.0

	Dose No.	% Survival	% Survival
Survival Results	Control	100	100
Test Date: <u>8/13/74</u>	1	100	100
	2	100	27
	3	100	45
	4	100	0
	5	80	0

	Dose	% Concentration	% Concentration
Concentrations of the test chemical required for mutagenicity tests	Plate Test	--	2.50
	$\frac{1}{4}$ 50% Survival	--	1.25
	$\frac{1}{2}$ 50% Survival	--	2.50
	Other	H 5.00	--
		L 2.50	

# VI. NON-ACTIVATION PLATE TESTS

DATE: 9-19-74

Test	Compound	Concentration/plate	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
			T-1	T-2	T-1	T-2	T-1	T-2
PC	EMS	0.05 ml undiluted chemical	>10 <sup>3</sup>	>10 <sup>3</sup>				
	QM	0.25 mg			56	43		
	NF	0.25 mg					25	31
SC	SALINE	-	3	0	3	4		
	DMSO	<10%					3	5

NOTE: PC = positive control  
 SC = solvent control  
 T-1 = trial 1  
 T-2 = trial 2  
 EMS = ethyl methanesulfonate  
 QM = quinacrine mustard  
 NF = nitrosofluorene  
 DMSO = dimethyl sulfoxide  
 (c) = contamination present

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NON-ACTIVATION PLATE TESTS

DATE: 9/19/74

Test	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
			T-1	T-2	T-1	T-2	T-1	T-2
TC	FDA 73-4	2.5%	2	0	3	2	2	1

NOTE: TC = test compound  
T-1 = trial 1  
T-2 = trial 2  
(c) = contamination present

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# VII. ACTIVATION PLATE TESTS

SPECIES: Mouse

DATE: 9-19-74

Test	Organ	Compound	Concentration/plate	TA-1535		TA-1537		TA-1538	
				T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 $\mu$ moles	>10 <sup>2</sup>	>10 <sup>2</sup>				
		AAF	1.25 mg			>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>
	Lu	DMNA	25 $\mu$ moles	7	3				
		AAF	1.25 mg			17	18	45	35
	T	DMNA	25 $\mu$ moles	(c)	5				
		AAF	1.25 mg			23	16	16	13
SC	-	DMNA	25 $\mu$ moles	3	4				
	-	AAF	1.25 mg			15	19	17	14
	-	Saline	-	3	4				
	-	DMSO	<10%			12	14	15	13

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 AAF = 2-acetylaminofluorene  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung

T = testes  
 T-1 = trial 1  
 T-2 = trial 2  
 DMSO = dimethyl sulfoxide  
 (c) = contamination present

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### ACTIVATION PLATE TESTS

SPECIES: Mouse

DATE: 9/19/74

Test	Organ	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
				T-1	T-2	T-1	T-2	T-1	T-2
TC	Li	FDA 73-4	2.5%	2	4	17	23	18	21
	Lu	FDA 73-4	2.5%	3	1	24	28	29	27
	T	FDA 73-4	2.5%	2	4	19	17	25	25

NOTE: TC = test compound  
Li = liver  
Lu = lung  
T = testes  
T-1 = trial 1  
T-2 = trial 2  
(c) = contamination present

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# ACTIVATION PLATE TESTS

DATE: 9-19-74

SPECIES: Rat

Test	Organ	Compound	Concentration/plate	TA-1535		TA-1537		TA-1538	
				T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 $\mu$ moles	>10 <sup>2</sup>	>10 <sup>2</sup>				
		AAF	1.25 mg			>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>
	Lu	DMNA	25 $\mu$ moles	2	3				
		AAF	1.25 mg			17	8	28	20
	T	DMNA	25 $\mu$ moles	3	6				
		AAF	1.25 mg			7	8	14	9
SC	-	DMNA	25 $\mu$ moles	3	4				
	-	AAF	1.25 mg			15	19	17	14
	-	Saline	-	3	4				
	-	DMSO	<10%			12	14	15	13
	-								

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 AAF = 2-acetylaminofluorene  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung

T = testes  
 T-1 = trial 1  
 T-2 = trial 2  
 DMSO = dimethyl sulfoxide  
 (c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: Rat

DATE: 9/19/74

Test	Organ	Compound	Concentration	TA-1535		TA-1537		TA-1538			
				T-1	T-2	T-1	T-2	T-1	T-2		
TC	Li	FDA 73-4	2.5%	3	4		30	14		27	25
	Lu	FDA 73-4	2.5%	7	9		27	42		35	30
	T	FDA 73-4	2.5%	4	1		42	22		29	19

NOTE: TC = test compound  
 Li = liver  
 Lu = lung  
 T = testes  
 T-1 = trial 1  
 T-2 = trial 2  
 (c) = contamination present

Project No. 2468

# ACTIVATION PLATE TESTS

SPECIES: Monkey

DATE: 9-19-74

Test	Organ	Compound	Concentration/plate	TA-1535		TA-1537		TA-1538	
				T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 $\mu$ moles	>10 <sup>2</sup>	>10 <sup>2</sup>				
		AAF	1.25 mg			>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>
	Lu	DMNA	25 $\mu$ moles	3	4				
		AAF	1.25 mg			10 <sup>-8</sup>		13	10
	T	DMNA	25 $\mu$ moles	3	3				
		AAF	1.25 mg			10	7	11	11
SC	-	DMNA	25 $\mu$ moles	3	4				
	-	AAF	1.25 mg			15	19	17	14
	-	Saline	-	3	4				
	-	DMSO	<10%			12	14	15	13

NOTE: PC = positive control  
SC = solvent and chemical controls  
AAF = 2-acetylaminofluorene  
DMNA = dimethylnitrosamine  
Li = liver  
Lu = lung

T = testes  
T-1 = trial 1  
T-2 = trial 2  
DMSO = dimethyl sulfoxide  
(c) = contamination present

Project No. 2468

ACTIVATION PLATE TESTS

SPECIES: Monkey

DATE: 9/19/74

Test	Organ	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
				T-1	T-2	T-1	T-2	T-1	T-2
TC	Li	FDA 73-4	2.5%	3	0	15	12	26	18
	Lu	FDA 73-4	2.5%	1	2	8	8	9	17
	T	FDA 73-4	2.5%	3	1	4	6	15	17

NOTE: TC = test compound  
 Li = liver  
 Lu = lung  
 T = testes  
 T-1 = trial 1  
 T-2 = trial 2  
 (c) = contamination present

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VIII. NON-ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS:  
POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 9-8-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	TA-1535	EMS	0.05 %	2.96	1,790	604.73
	TA-1537	QM	0.01 mg/ml	3.11	911	292.93
	TA-1538	NF	1.25 mg/ml	3.66	266	72.68
SC	TA-1535	SALINE	-	3.27	6	1.83
	TA-1537	SALINE	-	4.66	37	7.94
	TA-1538	DMSO	-	3.01	13	4.32

NOTE: PC = positive control  
 SC = solvent control  
 EMS = ethyl methanesulfonate  
 QM = quinacrine mustard  
 NF = nitrosofluorene  
 DMSO = dimethyl sulfoxide  
 (c) = contamination present

Project Number 2468



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NON-ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS

DATE: 9/8/74

Test	Indicator Strain	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
TC	TA-1535	FDA 73-4	H	3.23(99)	8	2.48
TC	TA-1535	FDA 73-4	L	3.12(95)	3	0.96
TC	TA-1537	FDA 73-4	H	8.68(186)	26	3.00
TC	TA-1537	FDA 73-4	L	7.70(165)	16	2.08
TC	TA-1538	FDA 73-4	H	4.34(144)	31	7.14
TC	TA-1538	FDA 73-4	L	4.32(144)	26	6.02

NOTE: TC = test compound  
H = high dose  
L = low dose  
(c) = contamination present  
( ) = percent survival

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IX. ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS:  
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE

DATE: 9-10-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	DMNA	100 $\mu$ moles/ml	4.11	4,449	1082.48
	Lu	DMNA	100 $\mu$ moles/ml	2.59	24	9.27
	T	DMNA	100 $\mu$ moles/ml	4.14	22	5.31
SC	-	DMNA	100 $\mu$ moles/ml	3.37	6	1.78
	-	SALINE	-	3.84	8	2.08

DATE: 9-23-74

Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	4.64	80	17.24
	Lu	AAF	1.25 mg/ml	5.76	10	1.74
	T	AAF	1.25 mg/ml	5.04	22	4.37
SC	-	AAF	1.25 mg/ml	5.24	41	7.82
	-	DMSO	-	6.01	41	6.82

DATE: 9-24-74 (Repeated Compounds)

Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	4.26	181	42.49
	Lu	AAF	1.25 mg/ml	2.04	7	3.43
	T	AAF	1.25 mg/ml	3.55	6	1.70
SC	-	AAF	1.25 mg/ml	1.46	24	16.44
	-	DMSO	-	2.85	37	12.98

NOTE: PC = positive control  
SC = solvent and chemical controls  
AAF = 2-acetylaminofluorene  
DMNA = dimethylnitrosamine  
Li = liver  
Lu = lung  
T = testes  
DMSO = dimethyl sulfoxide

(c) = contamination present

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ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS

SPECIES: Mouse

DATE: 9/10/74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
TC	Li	FDA 73-4	H	3.61 (94)	12	3.32
			L	3.22 (84)	17	5.28
	Lu	FDA 73-4	H	3.25 (85)	9	2.77
			L	2.76 (72)	4	1.45
	T	FDA 73-4	H	3.78 (98)	20	5.29
			L	3.64 (95)	13	3.57

DATE: 9/23/74

Strain TA-1537

TC	Li	FDA 73-4	H	5.15 (86)	13	2.52
			L	4.14 (69)	15	3.62
	Lu	FDA 73-4	H	4.35 (72)	8	1.84
			L	5.64 (94)	8	1.42
	T	FDA 73-4	H	4.53 (75)	27	5.96
			L	5.12 (85)	41	8.01

DATE: 9/24/74

Strain TA-1538

TC	Li	FDA 73-4	H	2.80 (98)	11	3.93
			L	2.34 (82)	36	15.38
	Lu	FDA 73-4	H	2.59 (91)	27	10.43
			L	3.03 (106)	35	11.55
	T	FDA 73-4	H	4.18 (147)	30	7.18
			L	2.28 (80)	47	20.61

NOTES: H = high dose  
L = low dose  
TC = test compound  
Li = liver  
Lu = lung  
T = testes  
(c) = contamination present  
( ) = percent survival

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**ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS:  
POSITIVE AND SOLVENT CONTROL RESULTS**

SPECIES: RAT

DATE: 9-13-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ ml x 10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	DMNA	100 $\mu$ moles/ml	3.83	1,342	350.39
	Lu	DMNA	100 $\mu$ moles/ml	4.34	9	2.07
	T	DMNA	100 $\mu$ moles/ml	3.39	15	4.43
SC	-	DMNA	100 $\mu$ moles/ml	3.14	5	1.59
	-	SALINE	-	4.13	7	1.70

DATE: 9-25-74

Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ ml x 10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	4.93	146	29.62
	Lu	AAF	1.25 mg/ml	5.15	64	12.43
	T	AAF	1.25 mg/ml	4.87	79	16.22
SC	-	AAF	1.25 mg/ml	6.30	110	17.46
	-	DMSO	-	6.93	67	9.67

DATE: 9-24-74

Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ ml x 10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	3.26	137	42.02
	Lu	AAF	1.25 mg/ml	3.18	33	10.38
	T	AAF	1.25 mg/ml	2.30	37	16.09
SC	-	AAF	1.25 mg/ml	1.46	24	16.44
	-	DMSO	-	2.85	37	12.98

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 AAF = 2-acetylaminofluorene  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung  
 T = testes  
 DMSO = dimethyl sulfoxide

(c) = contamination present

Project No. 2468



**BIONETICS**

ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS

SPECIES: Rat

DATE: 9/13/74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
TC	Li	FDA 73-4	H	3.29(80)	4	1.22
			L	2.80(68)	7	2.50
	Lu	FDA 73-4	H	3.79(92)	4	1.06
			L	3.23(78)	3	0.93
	T	FDA 73-4	H	3.47(84)	11	3.17
			L	3.64(88)	14	3.85

DATE: 9/25/74

Strain TA-1537

TC	Li	FDA 73-4	H	5.11(73)	38(c)	7.44
			L	4.73(68)	26	5.50
	Lu	FDA 73-4	H	5.13(74)	44	8.58
			L	5.05(73)	60	11.88
	T	FDA 73-4	H	5.63(81)	32	5.68
			L	4.95(71)	48	9.70

DATE: 9/24/74

Strain TA-1538

TC	Li	FDA 73-4	H	0.60(2)	11	18.33
			L	4.19(147)	58	13.84
	Lu	FDA 73-4	H	2.04(72)	43	21.08
			L	4.22(148)	55	13.03
	T	FDA 73-4	H	1.98(69)	15(c)	7.58
			L	3.39(119)	44	12.98

NOTES: H = high dose  
L = low dose  
TC = test compound  
Li = liver  
Lu = lung  
T = testes  
(c) = contamination present  
( ) = percent survival

Project No. 2468



BIONETICS

**ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS:  
POSITIVE AND SOLVENT CONTROL RESULTS**

SPECIES: MONKEY

DATE: 10-1-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	DMNA	100 $\mu$ moles/ml	5.33	1,600	300.19
	Lu	DMNA	100 $\mu$ moles/ml	5.91	10	1.69
	T	DMNA	100 $\mu$ moles/ml	5.91	5	0.85
SC	-	DMNA	100 $\mu$ moles/ml	4.24	9	2.12
	-	SALINE	-	5.24	9	1.72

DATE: 9-25-74

Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	3.90	95	24.36
	Lu	AAF	1.25 mg/ml	2.95	22	7.46
	T	AAF	1.25 mg/ml	5.29	28	5.29
SC	-	AAF	1.25 mg/ml	3.18	25	7.86
	-	DMSO	-	2.66	15	5.64

DATE: 10-4-74

Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
PC	Li	AAF	1.25 mg/ml	6.50	197	30.31
	Lu	AAF	1.25 mg/ml	6.87	86	12.52
	T	AAF	1.25 mg/ml	4.36	69	15.83
SC	-	AAF	1.25 mg/ml	4.90	84	17.14
	-	DMSO	-	5.55	71	12.79

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 AAF = 2-acetylaminofluorene  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung  
 T = testes  
 DMSO = dimethyl sulfoxide

(c) = contamination present

Project No. 2468



**BIONETICS**

Litton

ACTIVATION SUSPENSION TESTS  
WITH SALMONELLA INDICATOR STRAINS

SPECIES: Monkey

DATE: 10/1/74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 <sup>8</sup>	his+ Revertants/ ml	his+ Revertants/10 <sup>8</sup> Survivors
TC	Li	FDA 73-4	H	4.58(87)	2	0.44
			L	5.68(108)	2	0.35
	Lu	FDA 73-4	H	5.85(112)	0	--
			L	4.30(82)	7	1.63
	T	FDA 73-4	H	6.83(130)	3	0.44
			L	5.32(102)	0	--

DATE: 9/25/74

Strain TA-1537

TC	Li	FDA 73-4	H	3.62(136)	15	4.14
			L	4.82(181)	20	4.15
	Lu	FDA 73-4	H	3.98(150)	10	2.51
			L	5.04(189)	11	2.18
	T	FDA 73-4	H	3.99(150)	14	3.51
			L	4.60(173)	27	5.87

DATE: 10/4/74

Strain TA-1538

TC	Li	FDA 73-4	H	4.53(82)	45	9.93
			L	5.10(92)	75	14.71
	Lu	FDA 73-4	H	6.29(113)	56	8.90
			L	7.56(136)	87	11.51
	T	FDA 73-4	H	7.83(141)	54	6.90
			L	6.12(110)	84	13.73

NOTES: H = high dose  
L = low dose  
TC = test compound  
Li = liver  
Lu = lung  
T = testes  
(c) = contamination present  
( ) = percent survival

Project No. 2468



BIONETICS

X. NON-ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 9-16-74

Strain D4							
Test	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Convertants <sup>b</sup>		Convertants Per 10 <sup>5</sup> Survivors	
				Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
PC	EMS	1.0 %	6.94	189	271	27.23	39.05
SC	Saline	-	7.69	27	38	3.51	4.94

NOTE: PC = positive control  
 SC = solvent control  
 EMS = ethyl methanesulfonate

a = number x 10<sup>5</sup>  
 b = number at 10<sup>-1</sup> dilution  
 (c) = contamination present

Project No. 2468



BIONETICS

NON-ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 9/16/74

Strain D4							
Test	Compound	Concentration	Total Population Screened <sup>a</sup>	Number Convertants <sup>b</sup>		Convertants Per 10 <sup>5</sup> Survivors	
				Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
TC	FDA 73-4	H	7.16 (93)	25	20	3.49	2.79
		L	7.18 (93)	32	26	4.46	3.62

NOTE: TC = test compound  
 H = high dose  
 L = low dose  
 a = number x 10<sup>5</sup>  
 b = number at 10<sup>-1</sup> dilution  
 (c) = contamination present  
 ( ) = percent survival

Project No. 2468



**BIONETICS**

**XI. ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4:  
POSITIVE AND SOLVENT CONTROL RESULTS**

SPECIES: Mouse

DATE: 9-19-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Convertants <sup>b</sup>		Convertants Per 10 <sup>5</sup> Survivors	
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
PC	Li	DMNA	150 $\mu$ moles/ml	5.16	82	77	15.89	14.92
	Lu	DMNA	150 $\mu$ moles/ml	4.93	13	10	2.64	2.03
	T	DMNA	150 $\mu$ moles/ml	5.10	11	6	2.16	1.18
SC	-	DMNA	150 $\mu$ moles/ml	6.51	20	26	3.07	3.99
	-	SALINE	-	6.47	37	22	5.72	3.40

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung  
 T = testes

a = number x 10<sup>5</sup>  
 b = number at 10<sup>-1</sup> dilution  
 (c) = contamination present

Project No. 2468



**BIONETICS**

Litton

ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: Mouse

DATE: 9/19/74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Conyertants <sup>b</sup>		Conyertants Per 10 <sup>5</sup> Survivors	
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
TC	Li	FDA 73-4	H	1.82(28)	12	6	6.59	3.30
			L	(c)4.70(73)	16(c)	16	3.40	3.40
	Lu	FDA 73-4	H	2.77(43)	7	7	2.53	2.53
			L	4.88(75)	15	18	3.07	3.69
T	FDA 73-4		H	2.93(45)	13	19	4.44	6.49
			L	(c)5.23(81)	20	19	3.82	3.63

NOTE:

TC = test compound  
H = high dose  
L = low dose  
Li = liver  
Lu = lung  
T = testes  
a<sub>x</sub> = number x 10<sup>5</sup>  
b = number at 10<sup>-1</sup> dilution  
(c) = contamination present  
( ) = percent survival

Project No. 2468



BIONETICS

ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4:  
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Rat

DATE: 10-18-74

High Dose				Strain D4				
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Convertants <sup>b</sup>		Convertants Per 10 <sup>5</sup> Survivors	
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
PC	Li	DMNA	150 µmoles/ml	7.93	78	75	9.84	9.46
	Lu	DMNA	150 µmoles/ml	7.99	33	27	4.13	3.37
	T	DMNA	150 µmoles/ml	9.03	36 (c)	31 (c)	3.99	3.43
SC	-	DMNA	150 µmoles/ml	8.74	42 (c)	31 (c)	4.81	3.55
	-	SALINE	-	7.13 (c)	43 (c)	24	6.03	3.37

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung  
 T = testes

a = number x 10<sup>5</sup>  
 b = number at 10<sup>-1</sup> dilution  
 (c) = contamination present

Project No. 2468



BIONETICS

**ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4**

SPECIES: Rat

DATE: 10/18/74

High Dose				Strain D4				
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Conyertants <sup>b</sup>		Conyertants Per 10 <sup>5</sup> Survivors	
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
TC	Li	FDA 73-4	H	5.62 (79)	20	14	3.56	2.49
			L					
	Lu	FDA 73-4	H	5.94 (83)	21	19	3.54	3.20
			L					
T	FDA 73-4		H	5.85 (82)	19	24	3.25	4.10
			L					

NOTE:

TC = test compound  
H = high dose  
L = low dose  
Li = liver  
Lu = lung  
T = testes  
a<sub>x</sub> = number x 10<sup>5</sup>  
b = number at 10<sup>-1</sup> dilution  
(c) = contamination present  
( ) = percent survival

Project No. 2468



**BIONETICS**

ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4:  
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Rat

DATE: 9-26-74

Low Dose				Strain D4				
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Convertants <sup>b</sup>		Convertants Per 10 <sup>5</sup> Survivors	
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
PC	Li	DMNA	150 $\mu$ moles/ml	5.85	73	70	12.48	11.97
	Lu	DMNA	150 $\mu$ moles/ml	5.81	31	11	5.34	1.89
	T	DMNA	150 $\mu$ moles/ml	5.00	32	19	6.40	3.80
SC	-	DMNA	150 $\mu$ moles/ml	5.27	31	23	5.88	4.36
	-	SALINE	-	5.50	28	20	5.09	3.64

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung  
 T = testes

a = number x 10<sup>5</sup>  
 b = number at 10<sup>-1</sup> dilution  
 (c) = contamination present

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BIONETICS

**ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4**

SPECIES: Rat

DATE: 9/26/74

Low Dose				Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Conyertants <sup>b</sup>		Conyertants Per 10 <sup>5</sup> Survivors
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup> Try <sup>+</sup>
TC	Li		H				
		FDA 73-4	L	5.52 (100)	28	16	5.07 2.90
	Lu		H				
		FDA 73-4	L	5.24 (95)	19	0	3.63 --
	T		H				
		FDA 73-4	L	5.75 (105)	24	14	4.17 2.44

NOTE:

TC = test compound  
H = high dose  
L = low dose  
Li = liver  
Lu = lung  
T = testes  
a<sub>x</sub> = number x 10<sup>5</sup>  
b = number at 10<sup>-1</sup> dilution  
(c) = contamination present  
( ) = percent survival

Project No. 2468



**BIONETICS**

ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4:  
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: Monkey

DATE: 10-22-74

					Strain D4			
Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Convertants <sup>b</sup>		Convertants Per 10 <sup>5</sup> Survivors	
					Ade <sup>+</sup>	Try <sup>+</sup>	Ade <sup>+</sup>	Try <sup>+</sup>
PC	Li	DMNA	150 µmoles/ml	5.88	68	67	11.56	11.39
	Lu	DMNA	150 µmoles/ml	5.26	30	28	5.70	5.32
	T	DMNA	150 µmoles/ml	5.85	31	40	5.30	6.83
SC	-	DMNA	150 µmoles/ml	4.89	27	44	5.52	9.00
	-	SALINE	-	4.14	34	35	7.95	8.21

NOTE: PC = positive control  
 SC = solvent and chemical controls  
 DMNA = dimethylnitrosamine  
 Li = liver  
 Lu = lung  
 T = testes

a = number x 10<sup>5</sup>  
 b = number at 10<sup>-1</sup> dilution  
 (c) = contamination present

Project No. 2468



**BIONETICS**

**ACTIVATION SUSPENSION TESTS  
WITH SACCHAROMYCES INDICATOR STRAIN D4**

SPECIES: Monkey

DATE: 10/22/74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened <sup>a</sup>	Number of Conyertants <sup>b</sup> Ade <sup>+</sup> Try <sup>+</sup>	Conyertants Per 10 <sup>5</sup> Survivors Ade <sup>+</sup> Try <sup>+</sup>
TC	Li	FDA 73-4	H	5.38 (130) 23	18(c)	4.28
			L	7.30 (176) 28(c)	32(c)	3.84
	Lu	FDA 73-4	H	4.04 (98) 19	27	4.70
			L	5.88 (142) 28	27	4.76
T	FDA 73-4		H	4.07 (98) 30	17	7.37
			L	5.37 (130) 19	21	3.54

NOTE:

TC = test compound  
H = high dose  
L = low dose  
Li = liver  
Lu = lung  
T = testes  
a = number x 10<sup>5</sup>  
b = number at 10<sup>-1</sup> dilution  
(c) = contamination present  
( ) = percent survival

Project No. 2468



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## XII. SUMMARY OF TEST RESULTS

COMPOUND FDA 73-4A. Suspension Tests

Test <sup>a</sup>	Activation		Salmonella Reversion Frequencies ( $\times 10^{-8}$ )			Saccharomyces D4 Conversion Frequencies ( $\times 10^{-5}$ )	
	Species <sup>b</sup>	Organ <sup>c</sup>	TA-1535	TA-1537	TA-1538	Ade <sup>+</sup>	Try <sup>+</sup>
NA-PC	-	-	604.73	292.93	72.68	27.23	39.05
NA-NC	-	-	1.83	7.94	4.32	3.51	4.94
NA-H	-	-	2.48	3.00	7.14	3.49	2.79
NA-L	-	-	0.96	2.08	6.02	4.46	3.62
A-NC (-C)	-	-	2.08	6.82	12.98	5.72	3.40
A-NC (+C)	-	-	1.78	7.82	16.44	3.07	3.99
A-PC	M	Li	1082.48	17.24	42.49	15.89	14.92
A-PC	M	Lu	9.27	1.74	3.43	2.64	2.03
A-PC	M	T	5.31	4.37	1.70	2.16	1.18
A-H	M	Li	3.32	2.52	3.93	6.59	3.30
A-L			5.28	3.62	15.38	3.40	3.40
A-H	M	Lu	2.77	1.84	10.43	2.53	2.53
A-L			1.45	1.42	11.55	3.07	3.69
A-H	M	T	5.29	5.96	7.18	4.44	6.49
A-L			3.57	8.01	20.61	3.82	3.63

<sup>a</sup> NA = non activation  
NC = negative control  
PC = positive control  
A = activation  
H = high dose  
L = low dose

<sup>b</sup> M = mouse  
Mo = monkey  
R = rat

<sup>c</sup> Li = liver  
Lu = lung  
T = testes

(-C) = solvent control  
(+C) = chemical control

COMPOUND FDA 73-4

B. Plate Tests

Test <sup>a</sup>	Activation		Salmonella Responses		
	Species <sup>b</sup>	Organ <sup>c</sup>	TA-1535	TA-1537	TA-1538
NA-PC	-	-	+	+	+
NA-NC	-	-	-	-	-
NA-H	-	-	-	-	-
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	M	Li	+	+	+
A-PC	M	Lu	-	-	-
A-PC	M	T	-	-	-
A-H	M	Li	-	-	-
A-H	M	Lu	-	-	-
A-H	M	T	-	-	-

<sup>a</sup> NA = non activation  
 NC = negative control  
 PC = positive control  
 A = activation  
 H = high dose  
 L = low dose

<sup>b</sup> M = mouse  
 Mo = monkey  
 R = rat

<sup>c</sup> Li = liver  
 Lu = lung  
 T = testes

(-C) = solvent control  
 (+C) = chemical control

Project No. 2468

## SUMMARY OF TEST RESULTS

COMPOUND FDA 73-4A. Suspension Tests

Test <sup>a</sup>	<u>Activation</u>		<u>Salmonella Reversion Frequencies (x 10<sup>-8</sup>)</u>			<u>Saccharomyces D4 Conversion Frequencies (x 10<sup>-5</sup>)</u>	
	Species <sup>b</sup>	Organ <sup>c</sup>	TA-1535	TA-1537	TA-1538	Ade <sup>+</sup>	Try <sup>+</sup>
NA-PC	-	-					
NA-NC	-	-					
NA-H	-	-					
NA-L	-	-					
A-NC (-C)	-	-	1.70	9.67	12.98	6.03(5.09)	3.37(3.64)
A-NC (+C)	-	-	1.59	17.46	16.44	4.81(5.88)	3.55(4.36)
A-PC	R	Li	350.39	29.62	42.02	9.84(12.48)	9.46(11.97)
A-PC	R	Lu	2.07	12.43	10.38	4.13(5.34)	3.37(1.89)
A-PC	R	T	4.43	16.22	16.09	3.99(6.40)	3.43(3.80)
A-H	R	Li	1.22	7.44	18.33	3.56	2.49
A-L			2.50	5.50	13.84	5.07	2.90
A-H	R	Lu	1.06	8.58	21.08	3.54	3.20
A-L			0.93	11.88	13.03	3.63	0.00
A-H	R	T	3.17	5.68	7.58	3.25	4.10
A-L			3.85	9.70	12.98	4.17	2.44

<sup>a</sup> NA = non activation  
 NC = negative control  
 PC = positive control  
 A = activation  
 H = high dose  
 L = low dose

<sup>b</sup> M = mouse  
 Mo = monkey  
 R = rat

<sup>c</sup> Li = liver  
 Lu = lung  
 T = testes

(-C) = solvent control  
 (+C) = chemical control

Two sets of D4 controls are shown: High dose (no parentheses) and low dose (parentheses).

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COMPOUND FDA 73-4

B. Plate Tests

Test <sup>a</sup>	<u>Activation</u>		<u>Salmonella Responses</u>		
	Species <sup>b</sup>	Organ <sup>c</sup>	TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	R	L1	+	+	+
A-PC	R	Lu	-	-	-
A-PC	R	T	-	-	-
A-H	R	L1	-	-	-
A-H	R	Lu	-	-	-
A-H	R	T	-	-	-

<sup>a</sup> NA = non activation  
 NC = negative control  
 PC = positive control  
 A = activation  
 H = high dose  
 L = low dose

<sup>b</sup> M = mouse  
 Mo = monkey  
 R = rat

<sup>c</sup> L1 = liver  
 Lu = lung  
 T = testes

(-C) = solvent control  
 (+C) = chemical control



BIONETICS

# SUMMARY OF TEST RESULTS

COMPOUND FDA 73-4

## A. Suspension Tests

Test <sup>a</sup>	Activation		Salmonella Reversion Frequencies (x 10 <sup>-8</sup> )			Saccharomyces D4 Conversion Frequencies (x 10 <sup>-5</sup> )	
	Species <sup>b</sup>	Organ <sup>c</sup>	TA-1535	TA-1537	TA-1538	Ade <sup>+</sup>	Try <sup>+</sup>
NA-PC	-	-					
NA-NC	-	-					
NA-H	-	-					
NA-L	-	-					
A-NC (-C)	-	-	1.72	5.64	12.79	7.95	8.21
A-NC (+C)	-	-	2.12	7.86	17.14	5.52	9.00
A-PC	Mo	Li	300.19	24.36	30.31	11.56	11.39
A-PC	Mo	Lu	1.69	7.46	12.52	5.70	5.32
A-PC	Mo	T	0.85	5.29	15.83	5.30	6.83
A-H	Mo	Li	0.44	4.14	9.93	4.28	3.35
A-L			0.35	4.15	14.71	3.84	4.38
A-H	Mo	Lu	0.00	2.51	8.90	4.70	6.68
A-L			1.63	2.18	11.51	4.76	4.59
A-H	Mo	T	0.44	3.51	6.90	7.37	4.18
A-L			0.00	5.87	13.73	3.54	3.91

<sup>a</sup> NA = non activation  
NC = negative control  
PC = positive control  
A = activation  
H = high dose  
L = low dose

<sup>b</sup> M = mouse  
Mo = monkey  
R = rat

<sup>c</sup> Li = liver  
Lu = lung  
T = testes

(-C) = solvent control  
(+C) = chemical control

COMPOUND FDA 73-4

B. Plate Tests

Test <sup>a</sup>	<u>Activation</u>		<u>Salmonella Responses</u>		
	Species <sup>b</sup>	Organ <sup>c</sup>	TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	+	+	+
A-PC	Mo	Li	-	-	-
A-PC	Mo	Lu	-	-	-
A-PC	Mo	T	-	-	-
A-H	Mo	Li	-	-	-
A-H	Mo	Lu	-	-	-
A-H	Mo	T	-	-	-

<sup>a</sup> NA = non activation  
 NC = negative control  
 PC = positive control  
 A = activation  
 H = high dose  
 L = low dose

<sup>b</sup> M = mouse  
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<sup>c</sup> Li = liver  
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### XIII. INTERPRETATION AND CONCLUSIONS

Compound FDA 73-4, Potassium Sorbate, was examined for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained.

#### A. Salmonella typhimurium

##### 1. Plate Tests

At a concentration of 2.5% (w/v), FDA 73-4 did not induce reversion in any of the three indicator strains.

##### 2. Non-activation Suspension Tests

All tests were negative.

##### 3. Activation Suspension Tests

All tests were negative.

#### B. Saccharomyces cerevisiae

##### 1. Non-activation Suspension Tests

All tests were negative.

##### 2. Activation Suspension Tests

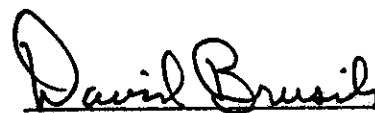
All tests were negative.

#### C. Additional Comments

None.

#### D. Conclusions

FDA 73-4, Potassium Sorbate, did not demonstrate any detectable genetic activity in the tests used in this evaluation.



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BIONETICS

APPENDIX

SUMMARY OF TESTS EVALUATING DMSO FOR GENETIC  
ACTIVITY IN SALMONELLA AND SACCHAROMYCES



BIONETICS

COMPOUND DIMETHYSULFOXIDE

A. Suspension Tests

Test	<u>Activation</u>		<u>Salmonella Reversion Frequencies (x 10<sup>-8</sup>)</u>		<u>Saccharomyces D4 Conversion Frequencies (x 10<sup>-5</sup>)</u>	
	Species <sup>a</sup>	Organ <sup>b</sup>	TA-1535	TA-1538	Ade <sup>+</sup>	Try <sup>+</sup>
<u>Non-activation</u>						
Control (-C)	-	-	0.74	0.88	32.51	4.34
High Dose <sup>c</sup>	-	-	1.91	1.05	28.32	2.95
Low Dose <sup>d</sup>	-	-	0.53	1.37	40.73	0.49
<u>Activation</u>						
Control (+C)	-	-	1.80	0.36	38.27	2.47
Control (-C)	-	-	1.43	1.04	37.12	2.64
High Dose <sup>c</sup>	M	Li	0.34	1.07	47.77	2.75
	M	Lu	0.59	0.58	36.29	1.39
	M	T	0.62	0.30	34.35	1.94
Low Dose <sup>d</sup>	M	Li	-	0.87	34.02	1.18
	M	Lu	0.43	3.14	42.30	1.40
	M	T	0.11	0.39	45.95	2.32

Note: (-C) = solvent control and (+C) = test chemical control without homogenate

a M = mouse  
Mo = monkey  
R = rat

b Li = liver  
Lu = lung  
T = testes

c Bacteria = 3%  
Yeast = 5%

d Bacteria = 4.5%  
Yeast = 2.5%

COMPOUND DIMETHYSULFOXIDE

B. Plate Tests

Test	<u>Activation</u>		<u>Salmonella Responses</u>		
	Species <sup>a</sup>	Organ <sup>b</sup>	TA-1535	TA-1537	TA-1538
<u>Non-activation</u>					
Control (-C)	-	-	-	-	-
Test compound (3%)	-	-	-	-	-
<u>Activation</u>					
Control (+C)	-	-	-	-	-
Control (-C)	-	-	-	-	-
Test compound (3%)	M	Li	-	-	-
	M	Lu	-	-	-
	M	T	-	-	-
	R	Li	-	-	-
	R	Lu	-	-	-
	R	T	-	-	-
	Mo	Li	-	-	-
	Mo	Lu	-	-	-
	Mo	T	-	-	-

Note: (-C) = solvent control and (+C) = chemical control without homogenate

a M = mouse  
Mo = monkey  
R = rat

b Li = liver  
Lu = lung  
T = testes